Claims 6 and 33 are vague and indefinite for depending upon a canceled claim

#3 Response:

Applicant has canceled claims 6 and 33 to clarify.

Claims 1, 7, 10, 20, 37 recite "two distinct fluorescent labels having the same spectral properties"

#4 Response:

Applicant has amended the claims to further clarify that the redundant labeling of cells is limited to two labels having different emission spectra, yet detected through the same spectral window.

"membrane dye" in claim 9 lacks antecedent basis in claim 7.

#5 Response:

Applicant has amended claim 9 to specifically refer to "at least two fluorescent labels" as "said label".

"cell fixative" in claims 12 and claim 18 lacks antecedent basis in claims 11 and claim 17.

#6 Response:

Applicant has deleted "cell" in claims 12 and 18 to further clarify the antecedent basis for fixative in claim 10 and 16.

Claims 11 and 17 are vague and indefinite because it is unclear how the buoyant density medium in which the cell of claim 10 or claim 16 is suspended further limits the stabilized cell of claim 10 or claim 16.

#7 Response:

Applicant has amended claims 11 and 17 to specify the stabilized cells are maintained in a medium where cells and medium have the same density, eliminating settling due to gravity (page 15 para 0134).

Claim Rejection under 35 USC § 103(a)

Claims 1, 2, 4, 10, 12, 14, 28, 29 and 31 are rejected over Davis as evidenced by Leif et al in view of Okada et al.

#8 Response:

Claim 1 is a process for producing a stabilized cell as an internal control comprising redundant labeling with two distinct fluorescent labels having the same spectral properties; permeabilizing the control cells; contacting with a fixative; removing excess fixative to promote long-term storage. An "internal control" as provided in the present invention must provide an internal control for the test system and a quantifiable means of cell recovery from the test system. So an internal control must assess how many target cells were actually retrieved and the volume of sample actually processed (page 7 para 0069).

Combining Davis, Leif et al and Okada et al could not provide the level of appreciation needed to successfully produce the internal control cells of the present application. Okada et al relies upon increasing detection sensitivity of a single signal by the identical labeling of the components (col 11 lines 8-11). This would ensure sensitivity for labeling of either or both components, but not confirm the presence of a control cell through dual labeling with two structurally and spectrally different fluorophores (page 10 para 0094), a problem not appreciated by the prior art. Further, pre-labeling with two different fluorophores having overlapping wavelengths, as in the present, invention reduces the probability of misclassifying a control cell as a target tumor cell, a factor not appreciated by Okada et al when the same fluorophore labels two separate cell components. Unlike Okada et al, detection in the present invention provides an extremely sensitive mechanism to quantify control cell recovery and assess target cell recovery.

Further, Okada et al provides for an immunological detection method only for immunochromatography. The labeling substances in Okada et al are those conventionally used in immunochemical assays (col 11, lines 3-7). Examples of labeling substances include alkali phosphatases and peroxidases; fluorescent substances like FITC and rhodamine (col 11, lines 5-11). The preparation

process of the present invention provides for a redundant labeling that is not limiting to the cell surface, and provides for the labeling of internal components (i.e. permeabilization step). This ensures the detection of only intact cells, without the possibility of redundantly-labeled cell debris resulting in an incorrect basis for quantitation of control cell count.

Additionally, the morphologic characteristics of the control cells in Davis were preserved through freeze-drying, an additional step not needed in the present invention. Combining the references would not address the previously unrecognized problem of ensuring, with high sensitivity, the ability to detect low numbers of intact control cells and distinguishing them from target cells, both by immunofluorescent labeling and by morphology without the reconstitution step.

With respect to fluorescent labeling, the present invention requires both excitation/emission spectra of the label to have minimal interference with target cell detection. For cell dyes, labels must be able to efficiently and uniformly stain cells in an irreversible way. They must have minimal leakage with minimal transfer of dye during storage (page 9, para 0093). Okada et al would need extensive experimentation to understand these issues. In fact, Okada et al does not limit immunochemical labeling to the criteria stated above (col 8 lines 31-37), suggesting that Okada et al does not appreciate the labeling issues of the present invention.

Thus, combining the prior art would not provide the motivation needed for the process and stabilized cells of the present invention. To further clarify this difference, applicant has amended independent claims 1, 10, 28 to limit the redundant labeling to the detection of a complete, intact control cell for quantitation, thereby removing any suggestion of the identical labeling of separate components on cell, cell debris, or cell membranes.

Claims 35 and 36 are rejected as unpatentable over Griwatz et al in view of Maples.

#9 Response:

The method taught in Griwatz et al does not provide for the level of detection needed in the present invention (page 1 para0012) where detection of a single

tumor cell in 3000-4000 total cells in circulation is needed. In order to obtain the level of sensitivity of the present invention, magnetic particles less than 200 nm, but large enough to be influenced by an externally applied magnet is required (page 7 para 0074). Further, magnetic particles in this size range are invisible to optical analytical techniques commonly used in rare cell analysis. Thus, the size allows for their Brownian energy to exceed their magnetic moment, avoiding particle attraction/repulsion when influenced by a magnetic field.

Griwatz et al. provides for the use of magnetic cell sorter (MACS) for cell separation (page 254, section 2.8). This method utilizes smaller particles which are separated by a 0.6 Tesla magnetic field. Thus, separation can only be completed by exposing the sample to steel wool fibers packed within the sample tube. Griwatz et al. would not appreciate an unrecognized problem that for rare cell detection levels of the present invention, the steel wool fibers could damage, and therefore affect, both the rare cells and the control cells in the sample. With internal gradient systems, the use of steel wool entraps non-magnetic components, for example non-target cells through capillary action in the vicinity of intersecting wires. These problems would be amplified when detecting select rare cancer cells that may be apoptotic or extremely fragile. In fact because these cells are extremely fragile, permeabilizing reagents significantly alter membrane soluble dyes, causing them the leach out of the cell and change morphological characteristics (page 9,para 0093). A surprising aspect of the present invention, not obvious from the prior art, is that the control cells are able to be prelabeled with membrane soluble dyes, permeabilized with miled detergents, fixed and stored, yet they retain their membrane label throughout the process up to six months.

Thus, the applicants submit the reasons set forth above for a lack of motivation to combine the prior art and the unexpected results embodied in the claims. To further prosecution, applicant has narrowed claims to limit the magnetic particles to colloidal magnetic particles between 90 and 200 nm size (page 7 para 0074) and thereby limit the separation to the use of an externally applied magnet to remove colloidal particles within a specific size.

Claims 35 and 36 are rejected as unpatentable over Griwatz et al in view of Maples as applied to claims 35 and 36 and further in view of Waggoner et al. and Haugland.

#10 Response:

As discussed above, the method for immunomagnetic rare cell separation taught in Griwatz et al (Response #9) would not enable the cell selection sensitivity required in the present invention. Therefore, further combining Waggoner et al and Haugland would not provide any further in site into the issues discussed (page 7 para 0074) in separating rare cells along with control cells.

Claims 1-4, 10-14, 28-31, and 34-38 are rejected as unpatentable over Griwatz et al and Maples and Waggoner et al and Haugland and further in view of Okada et al.

#11 Response:

As discussed above (Response #9), Okada et al does not provide the sensitivity for detecting intact control cells and distinguishing them from intact target cells (col 11 lines 8-11). Okada et al would also not have the same level of confidence in the determination of the state of the control cell, i.e. intact, debris, or broken cells.

The present invention provides an unexpected solution for this problem by providing a redundant labeled control cell having two different fluorescent labels with the same spectral properties (i.e. same detection window). Page 10; para 0094 discusses labeling with two different cellular components having two structurally and spectrally different fluorophores. In addition, this type of pre-labeling reduces the probability of misclassifying a control cell as a tumor cell.

Griwatz et al, as discussed above (Response #9), describes a cell selection process limited to MAC (magnetic cell sorter) whereby steel wool is in direct contact with the fluid sample to provide the magnetic field necessary to separate the target cells from the sample. Griwatz et al does not recognize the potential damage to the rare cells in the sample, particularly apoptotic or cancer cells. The present invention discloses a solution by using specific particles (50 to 200 nm)

in the presence of an externally applied magnet to separate both control and target cells from the sample, ensuring that these cells are not damaged by the separation process and providing the ability to analyze very low control and target cells.

Applicant has amended independent claims 1-10-28-35 to reflect this difference.

Claims 16-18 47 are unpatentable over Terstappen at al in view of Davis et al and Maples et al.

#12 Response:

Applicants suggest that the present invention provides inherent advantages not suggested by the combination of Terstappen et al, Davis et al, and Maples et al. Terstappen et al. teaches a method for enriching, detecting, and enumerating rare cells in a mixed population. Terstappen et al. does not consider the use of preserved stabilized control cells in the method, yet Terstappen et al does discuss the need for assessing false positive in determining the number of tumor cells per unit volume of biological sample (see WO99/41613 page 4; line 5-10). Terstappen et al suggests a need for having an internal control cells in the analysis of magnetic separations (page 12 lines 31 to page 13 line 8). Here, the efficiency, recovery, and purity of separation depends upon the factors such as the number of cells separated, receptor density, magnetic load per cell, non-specific binding, technique employed, nature of the vessel and surface, viscosity of the sample, and the separation device. Terstappen et al is unable to control for these variables and, in the examples 4-5-6 uses only patient controls as an external control, without the ability to compare efficiency, recovery, etc. of individual separations. External controls have been used for conventional low sensitivity flow or imaging assays of cells to detect systematic errors, but without the ability to detect random errors that cannot be detected with external controls alone. Unfortunately, external controls are not satisfactory when there is a need to address issues such as operator error, or with a standard that will act as a safe control from assay to assay, machine to machine, and laboratory to laboratory (page 2, para 0021). Any preferred control, used to address this issue, must be

consistently spiked into an unknown sample (internal control), and confidently recover the same controls for systematic comparisons, especially for rare cell analysis (page 4, para 0031). The lack of implementation of an internal control suggests that a combination of the prior art would not provide the level of control for accurately assessing the recovery circulating tumor cells in patient samples.

Davis does not teach the order of the claimed method steps, except after fixation (See Davis column 8, lines 45-49). Davis limits labeling to before or after drying, but always after fixation and reduction. The morphologic characteristics of the control cells in Davis were preserved through freeze-drying and re-hydrating before their use (see Response #8). Further, Davis is unable to retain the light scatter properties of cells without the addition of a reducing agent such as Schiff's base after fixation (col 4; lines 45-50) to prevent an increase in autofluorescence (col 7 line 39-43).

Maples et al uses control cells, reconstituted after lyophilization and labeled with antibodies either before or after reconstitution (page 6; lines 26-30 and page 7; lines 2-5)

The present invention does not incorporate a separate reduction (Schiff's base) or drying step. Thus, the present invention provides a means for pre-labeling and fixing without the need for subsequent reduction, providing a solution to a problem that previously was not obvious. The control cells remain preserved for 6 months without loss of their light scattering properties and without the expected problems of autofluorescence, providing an inherent advantage not appreciated in the prior art.

Further, Leif et al has been suggested in combination with Davis et al. While Leif has described the preservation of antigen-antibody complexes on leukocytes, inherent characteristics with labeling a circulating tumor cell as a control (SKBr-3 cells) would not be appreciated by combining Leif et al. For example, CTC's are more fragile cells and express both high and low antigen densities. Using this as an internal control cell in rare cell detection would create differences in antigen density and significantly alter determinations in recovery and efficiency. Accordingly, the present invention demonstrates that the antigen

density range of CULTURED cell lines available as internal control cells are similar to the range of tumor cells found in cancer patients (page 5; para 0039), providing a solution to a problem not appreciated by the combination of the prior art.

So while Terstappen et al. may provide a need to have an internal control cell in their analysis system, combining both the stability of the antigen-antibody complexes in leukocytes after cross-linking (Leif et al.) and the suggested improvement of differentially labeled control cells over fluorescent beads (Maples et al.) with the teachings from Davis would not solve the inherent problems, discussed above, to function in the Terstappen et al. system.

Applicant has amended the claims to reflect these specific differences not in the prior art.

Claims 16-18, 21, 22, 47-49 are unpatentable over Terstappen at al in view of Davis et al and Maples et al. in view of Griwatz et al, Brandes et al, and Hudziack et al.

#13 Response:

For the reasons discussed in Response #8 and #12, the combination of Terstappen et al, Davis et al, and Maples et al does not provide the level of appreciation that would make the present invention obvious. The rare cell detection system of Terstappen et al demonstrates an unmet need for an internal control cell that combining the fixed/reduced cells of Davis et al and the labeling of Maples et al would not provide the obvious extension for developing the internal control cell of the present invention, primarily because the prior art does not appreciate the ability to stabilize autofluorescence without a separate reduction (i.e. Schiffs base treatment). Further since limited to MAC and the inherent problems with an internal magnet as discussed above (Response #9), Griwatz et al could not be incorporated with the prior art to the present invention an obvious extension of the prior art.

Claims 16-18, 23, 47 and 50 are unpatentable over Terstappen at al in view of Davis et al and Maples et al. in view of Griwatz et al and Boffa et al.

#14 Response:

The combination of Terstappen et al, Davis et al, and Maples et al does not provide the level of appreciation that would make the present invention obvious as mentioned (see Response #8-#12-#13). The rare cell detection system of Terstappen et al demonstrates an unmet need for an internal control cell that, combining the fixed/reduced cells of Davis et al and the labeling of Maples et al, would not provide the obvious extension for developing the internal control cell of the present invention. This is because the present invention does not require treatment with Schiffs base to stabilize autofluorescence. Further since limited to MAC and the inherent problems with an internal magnet as discussed above (see Response #9), Griwatz et al could not be incorporated with the prior art as an obvious extension over the prior art.

Claims 16-18, 27, 47 and 54 are unpatentable over Terstappen at al in view of Davis et al and Maples et al. in view of Griwatz et al and Shih et al. (1994) and Shih (1999) and Silverman.

#15 Response:

Applicants refer to Responses #8-#9-#11-#12-#13-#14 associated with the reasons for a lack of motivation to combine the prior art and the demonstrate the unexpected results embodied in the claims.

Claims 1, 2, 4, 7, 8, 10, 16-18, 20, 28, 29, 31, 34, 35, 36, 37, 39, and 47 are unpatentable over Terstappen at al in view of Davis et al and Maples et al. in view of Okada.

#16 Response:

Applicants refer to Responses #8-#11-#12-#13-#14 associated with the reasons for a lack of motivation to combine the prior art and the demonstrate the unexpected results embodied in the claims.

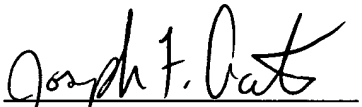
Claims 16-19 and 47 are unpatentable over Terstappen at al Davis et al and Maples et al. in view of Griwatz et al, Wagoner et al, and Haugland.

#17 Response:

Applicants refer to Responses #8-#9-#10-#11-#12-#13-#14 associated with the reasons for a lack of motivation to combine the prior art and the demonstrate the unexpected results embodied in the claims.

By the attached amendments, applicants have amended the claims to define the invention more particularly and distinctly so as to overcome the rejections and to patentably define the invention over the prior art. In view of these amendments and related discussions and arguments, it is respectfully urged that the rejections set forth in the December 02,2005 Office Action should be withdrawn and that this application be passed to issue. In the event the examiner has any comments or questions, the examiner is invited to telephone or e-mail applicants' undersigned representative at the number below.

Yours Respectfully,



Joseph F. Aceto
PTO Registration No. 50701

Telephone: (215) 346-8303
Facsimile: (215) 914-1117